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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/679,692	10/07/2003	Benjamin G. Davis	117-480	7864

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NIXON & VANDERHYE, PC
901 NORTH GLEBE ROAD, 11TH FLOOR
ARLINGTON, VA 22203

EXAMINER	
CHOWDHURY, IQBAL HOSSAIN	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 09/26/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/679,692	DAVIS, BENJAMIN G.	
	Examiner	Art Unit	
	Iqbal H. Chowdhury, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with th correspondenc address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 June 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-32* is/are pending in the application.

4a) Of the above claim(s) *24-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *03/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application is a non-provisional application of provisional application of 60/416,263.

The preliminary amendment filed on 6/15/2006 is acknowledged. Claims 1-32 are pending.

Applicant's election without traverse of Group I claims 1-23 and species, wherein methionine is modified at position M439 of SEQ ID NO: 2, in the communication filed on 6/15/2006 is acknowledged. Claims 24-32 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 1-23 are at issue and are present for examination.

Priority

Acknowledgement is made of applicants claim for priority of provisional application 60/416,263 of 10/7/2002.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 3/19/2004 is acknowledged. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Drawings

The drawings have been submitted on 10/7/2003 with this application.

Specification

Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

Applicant is reminded of the proper content of an abstract of the disclosure.

A patent abstract is a concise statement of the technical disclosure of the patent and should include that which is new in the art to which the invention pertains. If the patent is of a basic nature, the entire technical disclosure may be new in the art, and the abstract should be directed to the entire disclosure. If the patent is in the nature of an improvement in an old apparatus, process, product, or composition, the abstract should include the technical disclosure of the improvement. In certain patents, particularly those for compounds and compositions, wherein the process for making and/or the use thereof are not obvious, the abstract should set forth a process for making and/or use thereof. If the new technical disclosure involves modifications or alternatives, the abstract should mention by way of example the preferred modification or alternative.

The abstract should not refer to purported merits or speculative applications of the invention and should not compare the invention with the prior art.

Where applicable, the abstract should include the following:
(1) if a machine or apparatus, its organization and operation;
(2) if an article, its method of making;
(3) if a chemical compound, its identity and use;
(4) if a mixture, its ingredients;
(5) if a process, the steps.

In this case abstract has multiple paragraphs. Appropriate corrections are required.

Claim Objections

Claims 1 and 4-5 are objected to as encompassing non-elected subject matter. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1-23 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In the present instance, claims 1 and 5 recite the "a variant of" which is unclear as to the scope of mutants that are encompassed. In another words, how many changes can be made to a carbohydrate processing enzyme and still be "a variant of"?

Claims 1-23 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1 and 5 are indefinite in the recitation of the "equivalent to" with reference to carbohydrate processing enzyme, which is confusing. Does the term "equivalent" refer to a structural equivalent or functional equivalent of carbohydrate processing enzyme? If structural equivalent, does it mean mutants or variants of carbohydrate processing enzyme of SEQ ID NO: 2? If functional equivalent, does it mean the same activities of wild type carbohydrate processing enzyme or something else. Clarification is required. For examination purpose examiner will interpret it as any protein having carbohydrate processing or degrading enzyme activity.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-23 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a modified polypeptide of beta-glycosidase of SEQ ID NO: 2 comprising a mutation at M439 (methionine at 439 position) to C439 i.e. M439C, does not reasonably provide enablement for any carbohydrate processing or degrading or synthesizing enzyme or any variant of SEQ ID NO: 2 or any mutation in SEQ ID NO: 2 at any position or any mutation at any position equivalent to M439 or any polypeptide having one or more position substituted by any amino acid. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 1, 4 and 5 are so broad as to encompass any carbohydrate processing or degrading or synthesizing enzyme or any variant of SEQ ID NO: 2 or any mutation in SEQ ID NO: 2 at any position or any mutation at any position equivalent to M439 or any polypeptide having one or more position substituted by any amino acid. Claim 2 recites said polypeptide in which the mutation is selected to broaden the substrate specificity of the polypeptide compared to a polypeptide not so modified and claim 3 recites said polypeptide, wherein the mutation is an amino acid substitution. Claim 4 recites said polypeptide in which the polypeptide comprises: (i) SEQ ID NO: 2 having one or more of M439 substituted by cysteine, valine or alanine; or (ii) the amino acid sequence as defined in (b) or (c) having one or more of the amino acid residues equivalent to M439 substituted by cysteine, valine or alanine. Claim 5 recites a modified polypeptide having carbohydrate processing enzymatic activity, said polypeptide comprising an

amino acid sequence selected from (a) the amino acid sequence of SEQ ID NO: 2 comprising one or more mutations selected from the group consisting of M439C; (b) the amino acid sequence of a family 1 glycosyl hydrolase, comprising at least one mutation at an amino acid equivalent to M439 in SEQ ID NO: 2 wherein the amino acid is substituted by a C (cysteine) residue; and (c) a variant of (a) or (b) having carbohydrate processing enzymatic activity and comprising at least one mutation at an amino acid equivalent to M439 in SEQ ID NO:2 wherein the amino acid is substituted by a C (cysteine) residue. Claim 6 recites the polypeptide, wherein the C residue introduced by the mutation is chemically modified and claim 7 recites the polypeptide, wherein the C residue is modified so as to comprise a positively-charged group. Claim 8 recites the polypeptide, wherein the positively charged group is of formula -(CH2)n-N+R3, wherein n is a positive integer from 1 to 4 and each R, which may be the same or different, is H or a C1-C4 alkyl group. Claim 9 recites the polypeptide, wherein the positively charged group is -CH2CH2NMe3+ and claim 10 recites the polypeptide, wherein the C residue is modified so as to comprise a negatively-charged group. Claim 11 recites the polypeptide, wherein the negatively-charged group is of formula -(CH2)n-SO3 or -(CH2)n-COO, wherein n is a positive integer from 1 to 4 and claim 12 recites the polypeptide, wherein the negatively-charged group is of formula -CH2CH2-SO3. Claim 13 recites the polypeptide, wherein the C residue is modified so as to comprise an uncharged group and claim 14 recites the polypeptide, wherein the uncharged group is a C1-C4 alkyl group. Claim 15 recites the polypeptide, wherein the uncharged group is methyl and claim 16 recites the polypeptide, which further comprises a mutation of a catalytic nucleophilic residue of the active site. Claim 17 recites the polypeptide, wherein the further mutation is: (i) E387A or E387G in SEQ ID NO: 2 or (ii) substitution of

E387 with A or G in the amino acid sequence as defined in (b) or (c) of claim 1 and claim 18 recites the polypeptide, wherein the polypeptide has Glycosyl synthase, glycosyl hydrolase, and/or transglycosylase activity. Claim 19 recites the polypeptide, wherein the family 1 glycosyl hydrolase is Sulfolobus solfataricus 13-glycosidase and claim 20 recites the polypeptide according to claim 6, which further comprises a mutation of a catalytic nucleophilic residue of the active site. Claim 21 recites the polypeptide, wherein the further mutation is: (i) 387A or E387G in SEQ ID NO: 2 or (ii) substitution of E387 with A or G in the amino acid sequence as defined in (b) or (c) of claim 5 and claim 22 recites the polypeptide, wherein the polypeptide has Glycosyl synthase, glycosyl hydrolase, and/or transglycosylase activity. Claim 23 recites the polypeptide, wherein the family 1 glycosyl hydrolase is Sulfolobus solfataricus beta-glycosidase. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polypeptide having carbohydrate processing activity broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of only few carbohydrate processing enzymes.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims,

and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple point mutations or substitutions.

The specification does not support the broad scope of the claims which encompass any carbohydrate processing or degrading or synthesizing enzyme or any variant of SEQ ID NO: 2 or any mutation in SEQ ID NO: 2 at any position or any mutation at any position equivalent to M439 or any polypeptide having one or more position substituted by any amino acid because the specification does **not** establish: (A) regions of the protein structure which may be modified without effecting carbohydrate processing activity; (B) the general tolerance of polypeptide to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any polypeptide residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any carbohydrate processing or degrading or synthesizing enzyme or any variant of SEQ ID NO: 2 or any mutation in SEQ ID NO: 2 at any position or any mutation at any position equivalent to M439 or any polypeptide having one or more position substituted by any amino acid. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient

guidance, determination of polypeptide having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 1-5 and 18-19 are rejected under 35 U.S.C. 102(a) as being anticipated by Corbett et al. (Tailoring the substrate specificity of the beta-glycosidase from the thermophilic archaeon Sulfolobus solfataricus, FEBS Lett. 2001 Dec 14; 509(3): 355-60, see IDS). Corbett et al. disclose a modified polypeptide from Sulfolobus solfataricus, having carbohydrate processing activity, that is family 1 glycosyl hydrolase, which is 100% identical to SEQ ID NO: 2 of the instant application. Corbett et al. also teach a mutation at position M439 of the amino acid sequence of the protein as well as at positions E432 and W433. Corbett et al. also teach that the modified polypeptide has broadened substrate specificity, wherein the modification is performed by substitution mutation. Corbett et al. further teach that methionine at position 439 is mutated to cysteine residue (M439C) by substitution mutation. Corbett et al. furthermore teach glycosyl synthase, glycosyltransferase and glycosyl hydrolase, which is commonly known as beta-glycosidase. Therefore, Corbett et al. anticipate claims 1-5 and 18-19 of instant application.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 16-17 and 20-23 are rejected under 35 U.S.C. 103 (a) as being obvious over Corbett et al. (Tailoring the substrate specificity of the beta-glycosidase from the thermophilic archaeon Sulfolobus solfataricus, FEBS Lett. 2001 Dec 14; 509(3): 355-60, see IDS) in view of Withers et al. (US PGPUB 2003/0138880 A1, publication 7/24/2003, claim priority of 60/314,921 filed on 8/24/2001). Corbett et al. disclose a modified polypeptide from Sulfolobus solfataricus, having carbohydrate processing activity, that is family 1 glycosyl hydrolase, which is 100% identical to SEQ ID NO: 2 of the instant application. Corbett et al. also teach a mutation at position M439 of the amino acid sequence of the protein as well as at positions E432 and W433. Corbett et al. also teach that the modified polypeptide has broadened substrate specificity,

wherein the modification is performed by substitution mutation. Corbett et al. further teach that methionine at position 439 is mutated to cysteine residue (M439C) by substitution mutation. Corbett et al. furthermore teach glycosyl synthase, glycosyltransferase and glycosyl hydrolase, which is commonly known as beta-glycosidase. Corbett et al. do not teach a mutation at catalytic nucleophilic residue or specific mutants E387A or E387G.

However, Withers et al. teach a modified polypeptide from Sulfolobus solfataricus, same source as of instant application, having carbohydrate processing activity i.e. glycosidase, glycosyl synthase and glycosyltransferase. Withers et al. also teach mutation at catalytic nucleophilic residue of active site of said glycosidase enzyme. Withers et al. further teach mutation of E387A, E387G, or E387S. Withers et al. furthermore teach glycosyl synthase, glycosyltransferase and glycosyl hydrolyzing enzyme, which is commonly known as beta-glycosidase isolated from Sulfolobus solfataricus. Withers et al. in addition, teach chemically modifying polypeptide and polysaccharide molecules. Withers et al. further teach modification of nucleophilic residue of said enzyme results loses its ability to hydrolyze glycosidic bond but retaining glycosylation function by glycosylating of sugar acceptor molecules.

Since Withers et al. clearly taught a modification of glycosidase enzyme at catalytic nucleophilic residue (E387) of active site for altering substrate specificity of glycosidase enzyme by mutating glutamic acid (E) residue to alanine, glycine or serine residue, a skilled artisan would be motivated to utilize Withers et al. methods to modify a beta-glycosidase of Corbett et al. to produce an enzyme having altered nucleophilic interaction with substrate i.e. to reduce the hydrolyzing ability of the enzyme of glycosidic bond for substrate but retaining glycosylation function by glycosylating of sugar acceptor molecules.

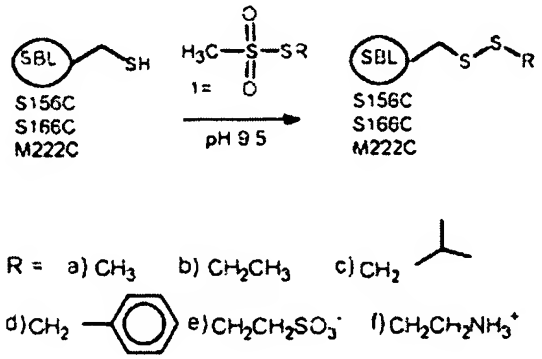
Therefore, it would have been obvious to one to ordinary skill in the art at the time of the invention was made to modify at catalytic nucleophilic residue (E387) of active site of glycosidase enzyme of Corbett et al. to produce an altered enzyme having altered nucleophilic interaction for substrate by mutating E387A or E387G by using the method of Withers et al. in the sequence of Corbett et al. to produce a modified beta-glycosidase to reduce the hydrolyzing ability of the enzyme of glycosidic bond for substrate but retaining glycosylation function by glycosylating of sugar acceptor molecules.

Claims 6-15 are rejected under 35 U.S.C. 103 (a) as being obvious over Corbett et al. (Tailoring the substrate specificity of the beta-glycosidase from the thermophilic archaeon Sulfolobus solfataricus, FEBS Lett. 2001 Dec 14; 509(3): 355-60, see IDS) in view of DeSantis et al (Site-directed mutagenesis combined with chemical modification as a strategy for altering the specificity of the S1 and S1' pockets of subtilisin Bacillus lentus, Biochemistry. 1998 Apr 28; 37(17): 5968-73). Corbett et al. disclose a modified polypeptide from Sulfolobus solfataricus, having carbohydrate processing activity, that is family 1 glycosyl hydrolase, which is 100% identical to SEQ ID NO: 2 of the instant application. Corbett et al. also teach a mutation at position M439 of the amino acid sequence of the protein as well as at positions E432 and W433. Corbett et al. also teach that the modified polypeptide has broadened substrate specificity, wherein the modification is performed by substitution mutation. Corbett et al. further teach that methionine at position 439 is mutated to cysteine residue (M439C) by substitution mutation. Corbett et al. furthermore teach glycosyl synthase, glycosyltransferase and glycosyl hydrolase,

which is commonly known as beta-glycosidase. Corbett et al. do not teach a chemically modification of mutated cysteine residue of M439C of modified beta-glycosidase.

However, DeSantis et al. teach site-directed mutagenesis combined with chemical modification as a strategy for altering the specificity of the S1 and S1' pockets of Subtilisin, an enzyme having hydrolase activity. DeSantis et al. also teach By combining site-directed mutagenesis with chemical modification through the incorporation of unnatural amino acid moieties, in the following manner: WT --> Cys-mutant + H3CSO2SR --> Cys-SR, where R may be infinitely variable. DeSantis et al. further teach introduction of a positive charged molecule, a negative charged molecule and uncharged molecule with altered enzymatic activity. DeSantis et al. also teach that chemical modified enzyme has dramatic enhancement of catalytic performance (122 fold decrease of Km). DeSantis et al. show the following scheme by which cysteine residue having –SH side chain could be chemically modified to alter the enzyme activity.

One of the ordinary skilled in the art would have been motivated to chemically modify modified-cysteine residue of beta glycosidase to enhance the specificity of the enzyme for substrate and enhancing the activity by creating a new active site environment.



It would have been obvious to one to ordinary skill in the art at the time of the invention was made to chemically modify modified-cysteine residue of M439C of beta-glycosidase of

Corbett et al. by using chemical modification methods as taught by DeSantis et al. to produce a chemically modified enzyme having altered substrate specificity with enhanced enzymatic activity.

Conclusion

Status of the claims:

Claims 1-23 are pending.

Claims 1-23 are rejected.

No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 703-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Iqbal Chowdhury, PhD, Patent Examiner
Art Unit 1652 (Recombinant Enzymes)
US Patent and Trademark Office
Rm. REM 2B69, Mail Box. 2C70
Ph. (571)-272-8137, Fax. (571)-273-8137

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